THEORIES OF ANTIBODY PRODUCTION

INTRODUCTION-THEORIES OF ANTIBODY PRODUCTION

- B- Lymphocytes recognize antigen and they differentiate into memory cells and antibody producing plasma cells.
- The theories of antibody production fall into two categories.
    - Selective theory
    - Instruction theory
- The above mentiontioned theories are supported by various other theories
    - Ehrlich's side chain theory (Selective theory)
    - Template Hypothesis (Instructive theory)
        - Direct template hypothesis
        - Indirect template theory
    - Natural selection theory
    - Clonal selection theory
    - Network theory
- Genetic Basis of antibody diversity
    - Germ line theory
    - Somatic mutation theory
- Isotype switching

SELECTIVE THEORY

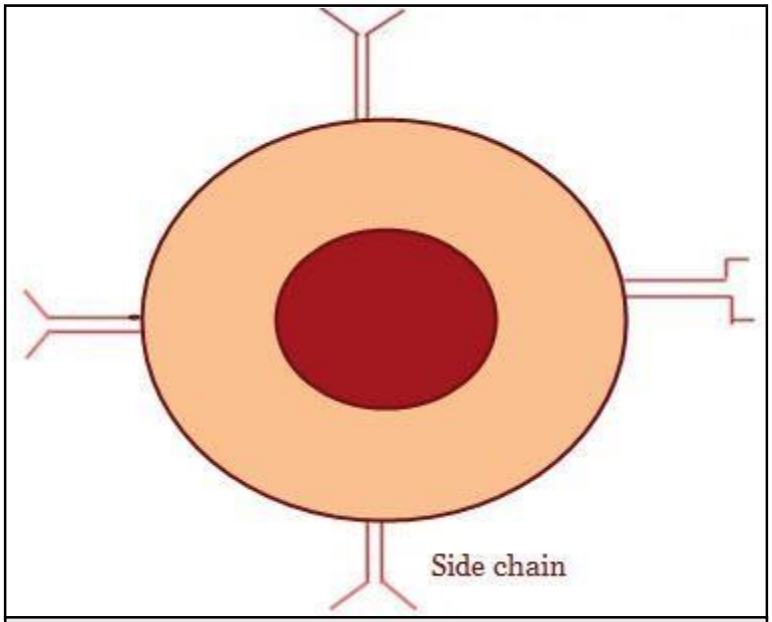
- According to this theory the immunocompetent cells have a restricted immunological range.
- Antigen stimulates the immunocompetent cells to selectively synthesize an antibody, i.e., all the genetic information ispresent in the cells before encountering the antigen.
- The cells were producing antibody at low level but antigenic stimulation results in rapid production of selective antibodies.

INSTRUCTIVE THEORY

- According to this theory, an immuno competent cell is competent to synthesize antibodies of any specificity.
- An antigen instructs the immunocompetent cells to synthesize complementary antibodies, i.e the cells did not have any genetic information earlier to the exposure to an antigen.
- There are various theories to support the above-mentioned theories.
    - Ehrlich's side chain theory (selective theory)
    - Template Hypothesis (Instructive theory)
        - Direct template hypothesis
        - Indirect template theory
    - Natural selection theory
    - Clonal selection theory
    - Network theory

EHRLICH'S SIDE CHAIN THEORY (SELECTIVE THEORY)

- Paul Ehrlich (1900) proposed this theory.
- Cells were considered to have surface receptors, which can bind with complementary 'side chains.
- These receptors are used for absorption of nutrients.
- When foreign substance or antigen enters into the body, they combine with the complementary surface receptor and inactivate them.



B cell contains different side chains (antibodies) with different specificity

- As a compensatory mechanism, there is over production of the same type of receptors and large numbers of them are liberated into the blood as circulatory antibodies.
- But this theory was abandoned when Land Steiner demonstrated that antibodies are also produced against various synthetic chemicals besides natural antigens.

TEMPLATE HYPOTHESIS (INSTRUCTIVE THEORY)

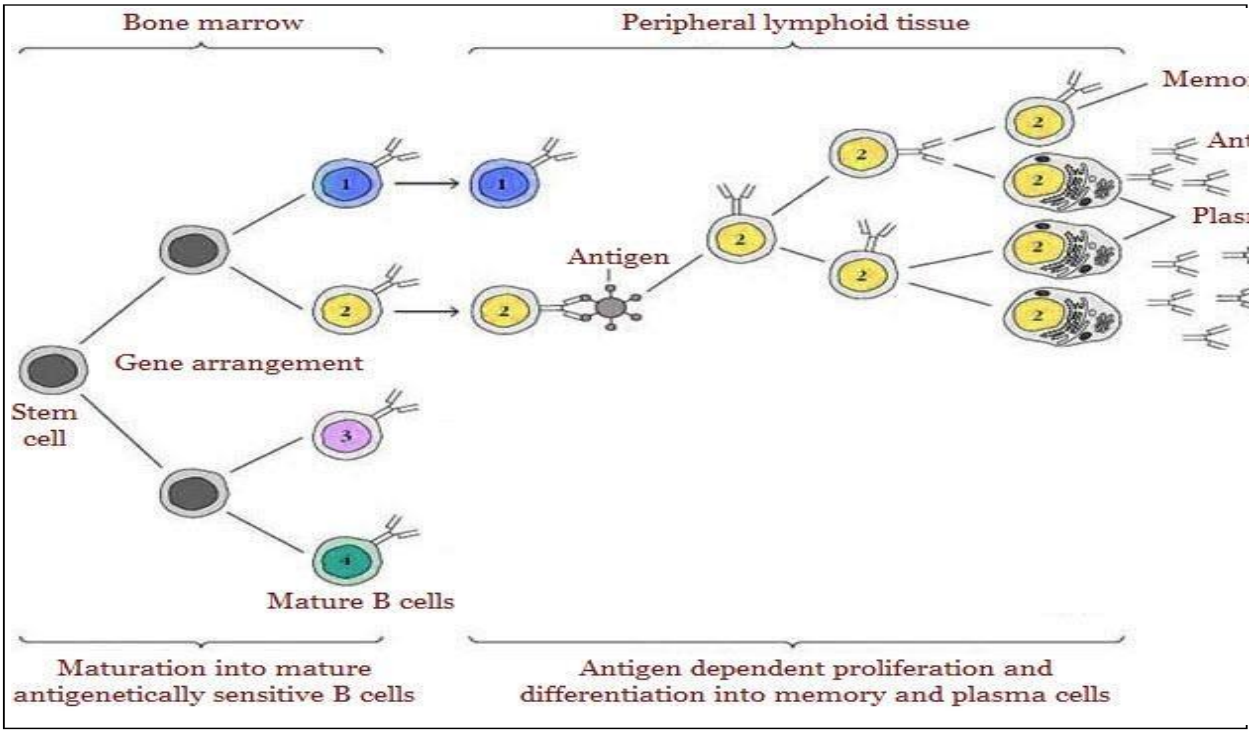
- Breint and Haurowitz proposed this theory in 1930.There are two types of template hypothesis
- Direct template hypothesis
    - According to this theory, antigen must enter the antibody forming cells and there are configuration changes in the antibody producing cells.
    - The antigen acts as a template (mould) for which complementary structure of antibodies are made by the cells.
    - Antibody is synthesized in direct contact with the antigen and then dissociate leaving the antigen free to act as template for further synthesis. Thus, mirror image of antigen is formed with its specificity.
- Indirect template theory
    - Burnet and Fenner (1949) proposed this instructive theory.
    - According to this theory antigen enter the antibody-producing cell and induce a heritable change.
    - Antigen alters the genome of the cell so that new template is formed which persist in the progeny cells (indirect template).
    - This explained specificity and the secondary response but it was abandoned when it was known that antibodies of different specificities had different amino acid sequence in their combining sites.

NATURAL SELECTION THEORY

- Jerne (1955) postulated natural selection theory.
- According to this about a million of globulin (antibody) with all specificities are formed in embryonic life.
- When an antigen enters, it combines with complementary antibody and the complex home to the antibody producing cells.
- It stimulates the cells to synthesize the same kind of antibody.
- But it failed to explain immunological memory.

CLONAL SELECTION THEORY

- Burnet (1957) proposed clonal selection theory for antibody synthesis.
- Medawar and Burnet received Nobel Prize in 1960.
- According to this theory, immune system recognize antigen by lymphocytes. The immunocompetent lymphocytes bear antibody receptors on their cell membrane with different specificity.
- An antigen stimulates a specific lymphocytes (B cells) clone to proliferate and differentiate into memory cells and plasma cells by binding to their antibody receptors.
- Thus, antigen with different determinants stimulates different clones of lymphocytes, which differentiate into different memory cells and antibody producing plasma cells.
- It is assumed that every immunocompetent lymphocyte (B cell) recognizes only one antigen and can synthesize only one type of immunoglobulin.
- The cells capable of reacting with different antigens were developed by somatic mutation.
- The cells that had immunological activity with self-antigen were eliminated during embryonic development and these clones are called forbidden clones. Their persistence would result in autoimmune disease.
- *The clonal selection theory is more accepted theory*.



NETWORK THEORY

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- Jerne postulated the network theory to explain the mechanism of regulation of antibody response.
- According to this, the variable region of immunoglobulin molecules differs in their amino acid sequence.
- The distinct amino acid sequences at their antigen binding sites and the adjacent path of the variable region are called *idiotypes*.
- The idiotype can act as antigen and produce anti-idiotype antibodies.
- These in turn produce antibodies, thus forming an idiotype network and regulate antibody production.
- Niels K. Jerne received Nobel Prize in 1984.

GENETIC BASIS OF ANTIBODY DIVERSITY

- An individual has capacity to produce at least 10^8 different antibody molecules. Such vast diversity require large number of genes.
- This is impossible as the human DNA molecule contain only 6.6 x 10^9 nucleotides.
- The genetic information for the synthesis of an immunoglobulin molecule is not present in a continuous array of codons.
- It is present in discontinuous stretches.
- The non-expressed intervening sequences or *introns* are present between the peptide coding sequences or *exons* within chromosomal DNA.
- Rearrangements of these sequences taken place during B cell differentiation and antibodies with various specificities are produced.
- Susumu Tonegawa of the Massachusetts Institute of Technology won Nobel Prize in 1987 for his contribution towards understanding the gene arrangement of antibody production.
- Immunoglobulin molecule is made up of V (variable) domain, C (constant) domain and J (joining) segments.
- V region of L-chain is encoded by V and J gene segments.
- V region of H-chain is encoded by V, D and J gene segments.
- In any particular species the number of C segments is limited and there need to be only one gene or a few genes for each constant region which are transmitted from generation to the generation in the germ line.
- The sequences in V domains are variable and body must produce large numbers of V_L and V_H to produce large diversified antibodies.
- The important theories for the origin of large number of V sequences are
    - Germ line theory
    - Somatic mutation theory

GERM LINE THEORY

- According to this theory germ cells carry structural genes for all the V_L and V_H chains.
- These genes would have arisen through gene duplication, mutation and selection.

SOMATIC MUTATION THEORY

- According to this theory germ cell contain limited number of genes, which become highly diversified through mutation in somatic cells during embryonic development resulting in differentiated clones of immuno competent cells that differ in genes.
- The number of genes involved is few. For example, in human V_K and V_λ genes it is only 3 and 5 respectively.
- Germ line V genes code for V domains that are specific for certain self-antigens e.g. histocompatibility antigen on cell surface and suppress the cells to produce antibodies against self-antigen.
- Mutation in the V gene code for antibodies that are specific but not against self-antigen.
- The somatic mutations usually result in greater affinity of the antibody for its antigen (affinity maturation).
- The rearrangements of DNA take place during B cell differentiation that make the diversity of antibody molecules produced.

ISOTYPE SWITCHING

- In case of re-exposure to some antigen, the IgM secreting cells will produce different antibodies like IgG or IgA with same specifies i.e. against the same antigen.
- Thus there is switch mechanism involve which enable the B-lymphocytes to utilize same variable genetic segment to produce one type (class) of antibody and again another type, all are directed against same antigen.
- In a resting B lymphocyte, one DNA segment code for all heavy chains except C_δ and possess a switch sequence (S) This S sequence enable any constant segment to combine with V_H-D_H-J_H .
- For example in first exposure to antigen, the B-lymphcytes secretes IgM due to V_H- D_H- J_H- C_μ gene. In subsequent exposure, there is switch over in S sequence and there is secretion of IgG because of V_H- D_H-J_H- C_γ gene, which translates into protein of IgG.
- The switch cause irreversible deformation of DNA and while rearrangements, the same variable segment is utilized with other constant segment to secrete specific class of antibodies.
- This happened, as there is deletion of μ and δ segments in original and change over to V_H-D_H-J_H-C_γ.

MONOCLONAL ANTIBODIES

- In general antibodies are produced by sensitized plasma cell and its clone in response to an antigen or antigenic determinants.

- Usually when infection takes place, polyclonal antibodies are produced as natural antigens have multiple epitopes or antigenic determinants.
- When a clone of plasma cells produce antibody against a single antigenic determinant, this antibody is known as monoclonal antibody.
- Monoclonal antibodies can be continuously produced by fusing antibody producing plasma cells to immortal myeloma cells and making hybrid cells, which have the capacity to grow for a prolonged time and this technology is known as Hybridoma technology.
- Hybridomas are somatic cell hybrids produced by fusing antibody forming spleen cells with myeloma cells.
- These hybrid cells retain the capacity of antibody producing spleen cells and indefinite multiplication of myeloma cells.
- This technology was first described by Kohler and Milstein (1975).

PRODUCTION OF MONOCLONAL ANTIBODIES

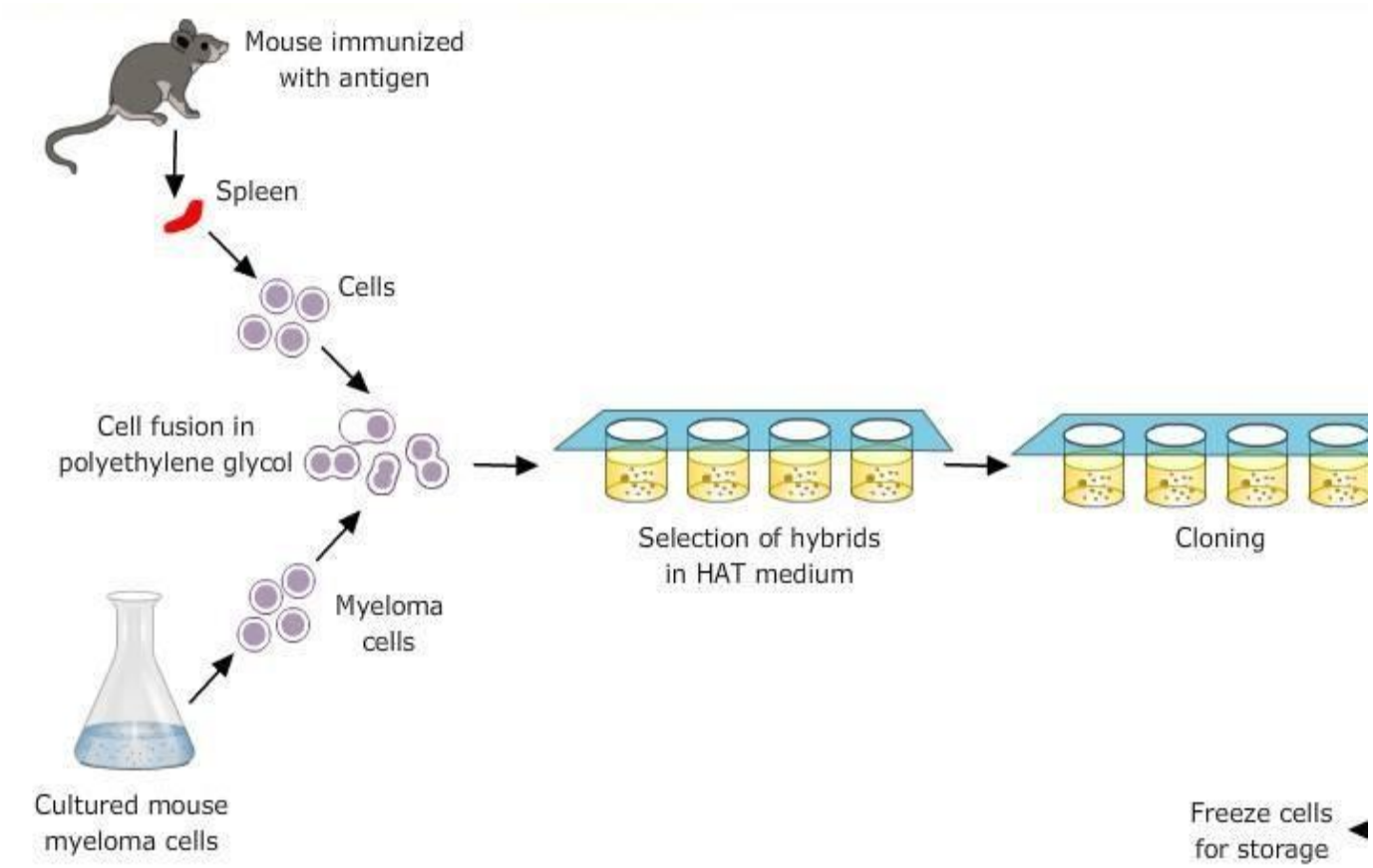
- This is done by different steps like generation of antibody producing plasma cells, fusion of plasma cells to the myeloma cells and re-cloning of cloned hybrid cells for specific antibody production.

Generation of antibody producing plasma cells

- By immunizing a mouse against the antigen of interest and repeating the process several times to ensure that it mount a good response.
- Mouse spleen is removed after 2 to 4 days of last administration of antigen and broken upto form almost single cell suspensions.

Fusion of plasma cells with myeloma cells:

- The spleen cells are suspended in cell culture medium together with the special mouse myeloma cells.
- It is usual to use myeloma cells that do not secrete immunoglobin as this may interfere the monoclonal antibody production (some examples are MOPC 21 BALB/C, MPC 11 BALB/C, SP2 /0BALB/C etc.).
- For induction of fusion, polyethylene glycol (PEG) is added to this mixture of cells (out of 2, 00,000 of spleen cells, one cell can be fused with one myeloma cells).
- The fused cell mixture is cultured for several days and the unfused spleen cells will die.
- There are three biosynthetic pathway for synthesis of nucleic acid (i.e. from Hypoxanthine, Thymidine and Uridine).
- Myeloma cells will survive but can be eliminated as they lack two enzymes namely hypoxanthine phosphoribosyl transferase (HGPRT) and thymidine kinase (Tk) but these are present in hybridoma cells. As myeloma cells lacks these two enzymes, they must use alternative pathway to convert uridine to nucleotides.
- The fused cells are allowed to grow into a culture media containing three compounds namely hypoxanthine, aminopterin and thymidine (called HAT medium).
- Aminopterin is a drug that prevents the myeloma cells to prevent making their own nucleotides from uridine and as a result they die soon. But the hybrid cells made from a myeloma and normal cells will grow since they possess hypoxanthine phosphoribosyl transferases and thymidine kinases thus utilize hypoxanthine and thymidine from culture medium to synthesize nucleic acids and survives – this pathway is called salvage pathway.
- Hybridoma cells divides rapidly on HAT medium doubling their number in 24-48 hours.
- On an average about 300-500 different hybrids can be isolated from a mouse spleen but all of them do not make antibody of interest.
- About 2-4 weeks of cultures growing cells can be seen and supernatant fluid should be checked for the presence of antibody.
- In a fusion experiment, about 50,000 myeloma cells are seeded per well in a culture plate and one hybrid cell can be obtained from every three wells.
- Clones are sub cloned to ensure that are it is single clone producing desired antibody or monoclonal antibody and that are grown in mass culture.
- Unfortunately, antibody producing hybrid cells lose their ability for culturing several times. So hybrids cells stock is aliquotinto small volume and preserved frozen for future use.



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APPLICATION

- Immunodiagnostics
  - This is used for specific diagnosis of several viral, bacterial and parasitic diseases.
  - For example, Mab based CELISA kits are widely used in antibody detection against blue tongue disease in sheep.
- Tumor diagnosis and therapy
  - Monoclonal antibodies (Mabs) are used for tumor diagnosis (using tumor specific MAbs) by imaging technique and also for immunotherapy.
- HLA typing

- Anti HLA Mabs are more suitable for forensic study and HLA typing.
- Being homogenous any analysis using MAb require short incubation period.